

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings of claims in the application:

**Listing of Claims:**

- 1-6. (Canceled)
7. (Previously Amended) A method for the absolute quantification of a target nucleic acid in a sample comprising the steps of:
- (a) determining the amplification efficiencies of the target nucleic acid and of an internal or external standard under defined amplification conditions by:
    - (i) preparing a dilution series of the target nucleic acid and a dilution series of the internal or external standard;
    - (ii) amplifying the target nucleic acid and the internal or external standard under defined reaction conditions and measuring the amplification in real-time;
    - (iii) setting a defined signal threshold value;
    - (iv) determining, for each dilution of the target nucleic acid and for each dilution of the internal or external standard, the cycle number at which the signal threshold value is exceeded;
    - (v) determining a non-linear continuously differentiable function of a logarithm of the copy number of target nucleic acid and the internal or external standard used for the amplification as a function of the cycle number at which the signal threshold value is exceeded; and
    - (vi) calculating the amplification efficiency of the target nucleic acid and the internal or external standard from said non-linear continuously differentiable function;
  - (b) amplifying the target nucleic acid contained in the sample and the internal or external standard under said defined reaction conditions;

- (c) measuring the amplification of the target nucleic acid and that of the internal or external standard in real time; and
- (d) calculating the original copy number in the sample by correcting the copy number derived from step (c) with the amplification efficiencies determined in step (a).

8. (Previously Amended) A method for quantification of a target nucleic acid in a sample relative to a reference nucleic acid comprising the steps of:

- (a) determining the amplification efficiencies of the target nucleic acid and of the reference nucleic acid under defined amplification conditions by:
  - (i) preparing a dilution series of the target nucleic acid and a dilution series of the reference nucleic acid;
  - (ii) amplifying the target nucleic acid and the reference nucleic acid under defined reaction conditions and measuring the amplification in real-time;
  - (iii) setting a defined signal threshold value;
  - (iv) determining, for each dilution of the target nucleic acid and for each dilution of reference nucleic acid, the cycle number at which the signal threshold value is exceeded;
  - (v) determining a non-linear continuously differentiable function of a logarithm of the copy number of target nucleic acid and the reference nucleic acid used for the amplification as a function of the cycle number at which the signal threshold value is exceeded; and
  - (vi) calculating the amplification efficiency of the target nucleic acid and the reference nucleic acid from said non-linear continuously differentiable function;
- (b) amplifying the target nucleic acid contained in the sample as well as the reference nucleic acid contained in the sample under said defined amplification conditions;
- (c) measuring the amplification of the target nucleic acid and that of the reference nucleic acid in real time; and

- (d) calculating the original ratio of target nucleic acid and reference nucleic acid in the sample by correcting the ratio derived from step (c) with the amplification efficiencies determined in step (a).
9. (Previously Amended) A method for quantification of a target nucleic acid relative to a reference nucleic acid and standardized with a calibrator sample comprising the steps of:
- (a) preparing a common or two separate dilution series of target nucleic acid and reference nucleic acid;
  - (b) amplifying the various dilutions of target nucleic acid and reference nucleic acid under defined reaction conditions, and measuring the amplification of the nucleic acids in real-time;
  - (c) setting defined signal threshold values for the target nucleic acid and reference nucleic acid;
  - (d) determining the cycle numbers  $C_p$  at which the signal threshold values defined for the target nucleic acid and reference nucleic acid are exceeded in each dilution;
  - (e) determining a continuously differentiable function of the  $C_p$  values determined in step d) as a function of the logarithm of the amounts used of target nucleic acid and determining a continuously differentiable function of the  $C_p$  values determined in step d) as a function of the logarithm of the amounts used of reference nucleic acid;
  - (f) determining the  $C_p$  values of the target nucleic acid and reference nucleic acid in a sample to be analysed as well as in a calibrator sample;
  - (g) assigning the  $C_p$  values measured in step f) to particular values of the functions determined in step e);
  - (h) calculating the quotients of the function values from step g) of the target nucleic acid and reference nucleic acid for the sample to be analysed as well as for the calibrator sample; and
  - (i) determining the ratio of the two quotients from step h) as a measure of the original amount of target nucleic acid contained in the sample to be analysed.

10. (Previously Amended) A method for quantification of a target nucleic acid relative to a reference nucleic acid and standardized with a calibrator sample comprising the steps of:
- (a) preparing a common or two separate dilution series of target nucleic acid and reference nucleic acid;
  - (b) amplifying the various dilutions of target nucleic acid and reference nucleic acid under defined reaction conditions, and measuring the amplification of the nucleic acids in real-time;
  - (c) setting defined signal threshold values for the target nucleic acid and reference nucleic acid;
  - (d) determining the cycle numbers  $C_p$  at which the signal threshold values defined for the target nucleic acid and reference nucleic acid are exceeded in each dilution;
  - (e) determining a continuously differentiable function of the logarithm of the amounts used of target nucleic acid as a function of the  $C_p$  values determined in step d) and determining a continuously differentiable function of the logarithm of the amounts used of reference nucleic acid as a function of the  $C_p$  values determined in step d);
  - (f) determining the  $C_p$  values of the target nucleic acid and reference nucleic acid in a sample to be analysed as well as in a calibrator sample;
  - (g) assigning the  $C_p$  values measured in step f) to particular values of the functions determined in step e);
  - (h) calculating the quotients of the function values from step g) of the target nucleic acid and reference nucleic acid for the sample to be analysed as well as for the calibrator sample; and
  - (i) determining the ratio of the two quotients from step h) as a measure of the original amount of target nucleic acid contained in the sample to be analysed.
11. (Previously amended) The method of claim 10, wherein the continuously differentiable functions from step e) are determined with a polynomial fit.

12. (Previously Amended) The method of claim 10, wherein the amplified nucleic acids are detected with at least one fluorescently-labeled hybridization probe.
13. (Previously Amended) The method of claim 12, wherein the amplified nucleic acids are detected with FRET hybridization probes, molecular beacons, or TAQMAN<sup>®</sup> probes.
14. (Currently Amended) The method of claim 10, wherein the amplified nucleic acids are Currently detected with a DNA-binding dye.